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Differential housing and novelty response: Protection and risk from locomotor sensitization

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Abstract

High novelty seeking increases the risk for drug experimentation and locomotor sensitization. Locomotor sensitization to psychostimulants is thought to reflect neurological adaptations that promote the transition to compulsive drug taking. Rats reared in enrichment (EC) show less locomotor sensitization when compared to rats reared in isolation (IC) or standard conditions (SC). The current research study was designed to test if novelty response contributed locomotor sensitization and more importantly, if the different housing environments could change the novelty response to protect against the development of locomotor sensitization in both adolescence and adulthood.

Experiment 1: rats were tested for their response to novelty using the inescapable novelty test (IEN) and pseudorandomly assigned to enriched (EC), isolated (IC), or standard (SC) housing conditions for 30 days. After housing, they were tested with IEN. Rats were then administered amphetamine (0.5 mg/kg) or saline and locomotor activity was measured followed by a sensitization test 14 days later. Experiment 2: rats were tested in the IEN test early adulthood and given five administrations of amphetamine (0.3 mg/kg) or saline and then either stayed in or switched housing environments for 30 days. Rats were then re-tested in the IEN test in late adulthood and administered five more injections of their respective treatments and tested for locomotor sensitization.

Results indicate that IC and SC increased the response to novelty. EC housing decreased locomotor response to amphetamine and saline, and SC housing increased the locomotor response to amphetamine. Mediation results indicated that the late adult novelty response fully mediates the locomotor response to amphetamine and saline, while the early adulthood novelty response did not.

Conclusions—Differential housing changes novelty and amphetamine locomotor response. Novelty response is altered into adulthood and provides evidence that enrichment can be used to reduce drug vulnerability.

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Keywords

DIFFERENTIAL REARING/HOUSING; ENRICHMENT; NOVELTY AND SENSATION SEEKING; AMPHETAMINE; SENSITIZATION; MEDIATION AND PREDICTION

1. Introduction

The transition to drug dependence has been characterized as a process that involves several phases (Koob & Le Moal, 1997, 2001; Koob & Volkow, 2009; Kreek & Koob, 1998). Numerous reports have observed that high novelty and sensation seeking individuals are more vulnerable to the initiation of drug use (Blanchard et al., 2009; Donohew et al., 1999; Martin et al., 2002; Zuckerman, 1994). Novelty and sensation seeking reaches maximal expression during adolescence (Arnett, 1992); a time when drug experimentation is also elevated (Arnett, 1992). Therefore, animal models of novelty response are widely used preclinical models for understanding the relationship between individual differences in novelty response and drug-use vulnerability.

One widely used animal model to examine the relationship between novelty and drug use examines the response to inescapable novelty. Piazza and colleagues (1989) categorized rats as either high (HR) or low (LR) novelty responders based on their amounts of locomotor activity in an inescapable novel environment. HR rats are more sensitive than LR rats to amphetamine-induced locomotor activity (Exner and Clark, 1993; Hooks et al., 1991; Hooks et al., 1992) and to amphetamine self-administration (Marinelli, 2005; Piazza et al., 1989; Pierre and Vezina, 1997). The difference between HR and LR rats is most robust at low unit doses (Cain et al., 2008; Kabbaj, 2006; Piazza et al., 1989). Numerous studies have demonstrated that the response to inescapable novelty predicts vulnerability to the early phases of drug use (Kabbaj, 2006; Marinelli, 2005; Piazza et al., 1989), prior to the development of compulsive drug taking (Belin et al., 2011; Blanchard et al., 2009).

While this model predicts the binge-intoxication stage of drug use, it is not clear what, if any, factors can alter this predisposition to novelty-seeking or the response to drugs of abuse. Therefore, the current experiments were designed to test if the differential housing manipulation will alter the novelty response and the response to amphetamine.

Understanding if the environment can alter the response to novelty and, subsequently the response to amphetamine will enable development of interventions to reduce sensitivity to the reinforcing effect of psychostimulants during the development of hyperactivity.

While a variety of methods examining human sensation seeking and drug use have been employed, these studies frequently use novelty seeking or sensation seeking as continuous variables in multiple regression analyses (Donohew et al., 1991; Roberti, 2004; Zuckerman, 1994). The animal models for novelty seeking are also measured on a continuous scale. However, the scores traditionally are dichotomized into high and low groups by a median split (Marinelli, 2005). The median split is problematic because it assumes the members of each group are similar and the members of different groups are categorically different, neither of which may be true. Further, the median split discards the precision of the original continuous measure, and may underestimate the relationship and decrease statistical power

(Cohen, 1983; Humphreys, 1974; Irwin et al., 2003; Maxwell et al., 1993). The current experiments used mediation regression analyses to analyze how novelty was changed and predicts amphetamine-induced locomotor activity. These analyses allow for the detection of more subtle differences changed by housing condition, because we can determine a change from a known baseline. Detecting a change in novelty response when novelty response is categorized requires a shift from one category to another category, which may not occur, despite a change in novelty response. The precision in measurement ensures more accurate analyses and relationships and precise descriptions of said relationships (Bissonnette et al., 1990a; Bissonnette et al., 1990b; Cohen, 1968).

In the differential housing paradigm, rats are raised in different environments from the post-weaning period through mid to late adolescence. In the enriched environment, rats receive daily handling and are raised in a large cage with several other rats and novel objects. Each of these factors are critical elements that create the enriched environment (Renner and Rosenzweig, 1987). Rats in the isolated environment are reared individually without novel objects or contact with other rats or the experimenters. The differential housing environments result in numerous neurobiological differences including, (Green and Greenough, 1986; Renner and Rosenzweig, 1987) changes within brain regions in the mesolimbic dopamine system that contribute to the response to novelty and drugs of abuse (Bardo et al., 2013; Green and Greenough, 1986; Melendez et al., 2004; Rahman and Bardo, 2008; Renner and Rosenzweig, 1987; Zhu et al., 2005).

The mesolimbic alterations that result from differential housing contribute to robust differences in novelty response. Post-weaning enrichment decreases locomotor activity in an inescapable novel environment (Fuller, 1967; Lore, 1966; Simpson and Kelly, 2011). In addition, enriched rats approach novel stimuli more quickly, and decrease responding to novel stimuli faster than do isolated rats (Zimmermann et al., 2001). Enrichment also reduces the locomotor response to low doses of psychostimulants (Bardo et al., 1995; Green et al., 2002). Enrichment decreases acute amphetamine-induced hyperactivity when compared to isolation (Bardo et al. 1995; Cain, Mersmann, Gill, & Pittenger, 2012; Gill, Arnold, & Cain, 2012). Enrichment also decreases psychostimulant-induced sensitization across a range of psychostimulants when compared to isolation (Bardo et al., 1995; Cain et al., 2012; Coolon and Cain, 2009; Smith et al., 1997; Wooters et al., 2011).

Given the well established effect of housing in an enriched environment on the response to psychostimulants, the ability of enrichment to function as an intervention following exposure to psychostimulants has been examined. Adult mice placed in enrichment following group housing had a decrease in cocaine-induced sensitization, but only after 30 days of enrichment (Solinas et al., 2008). This suggests that enrichment during adulthood may be able to decrease the locomotor response to psychostimulants, when enrichment is used as an intervention. However, research has not attempted to manipulate the novelty response to change the development of amphetamine-induced locomotor sensitization.

Therefore, the current experiments examined if differential housing, during the post-weaning period or in adulthood following psychostimulant exposure, can alter the response to novelty and the response to amphetamine. Generally, we hypothesized that enrichment would reduce

the response to inescapable novelty and would therefore decrease the response to amphetamine. Conversely, we predicted that isolation housing would increase the inescapable novelty response and the response to amphetamine. We also predicted the adult novelty response is stable and will not be significantly changed by housing condition. Interestingly, the response to inescapable novelty does not predict the transition from controlled drug use to compulsive drug use (Belin et al., 2008; Belin et al., 2011), but enrichment reliably decreases psychostimulant sensitization. Therefore, the current experiments also examined amphetamine-induced sensitization to determine if differential housing or novelty seeking is the better predictor of amphetamine-induced sensitization.

2. Materials and Methods

2.1 Animals

Male Sprague-Dawley rats (Charles River, Portage, MI, USA) arrived in the laboratory at 21 days of age and were housed in one of three differential housing conditions: enriched (EC), isolated (IC), or standard (SC). The colony room operated on a 12-hr light-dark cycle and was maintained at approximately 22° C, with humidity ranging from approximately 30–45%. All behavioral tests were conducted during the light portion of the cycle. All procedures conducted and research reported was in accordance with the Institutional Animal Care and Use Committee at Kansas State University, and complied with NIH guidelines (National Research Council (US) Committee for the Update of the Guide for the Care and Use of Laboratory Animals, 2011).

2.2 Differential Housing Conditions

Both Experiments utilized differential housing conditions, enriched (EC), isolated (IC), and standard (SC). Our EC, IC, and SC environmental conditions have been explained previously articles (Arndt et al., 2014; Arndt et al., 2015; Cain et al., 2012; Gill et al., 2012). Briefly, rats in the EC were housed in a large metal cage (60×120×45 cm) that was lined with paper pulp bedding. The EC cage contained 8–12 other rats and contained 14 objects (toys, PVC pipe). To maintain novelty, seven of the 14 objects were changed daily and all objects were changed two times weekly. The EC rats were also handled during the 30 day housing periods for ~1min per day. The IC rats were housed in hanging metal cages (17×24×20 cm), were not handled throughout the 30 day housing periods, and did not have access to novel objects or other rats. The SC rats were housed in pairs in standard shoebox cages (20×43×20 cm) with the same bedding as the EC rats. The SC rats did not have access to objects and were not handled during the 30 day housing periods. In Experiment 1, rats were placed in the EC, IC, or SC condition on postnatal day (PND) 22 and remained in this housing for the duration of the experiment. In Experiment 2, rats were placed in the EC or IC condition on Day 21 and housed for 30 days. After five administrations of amphetamine or saline, they stayed in their original housing condition or were switched to the EC or IC condition. Rats remained in the new housing assignment for an additional 30 days. After the additional 30 days, another round of behavioral testing commenced and the rats remained in their respective conditions for the duration of the experiment.

2.3 Apparatus

Locomotor activity during the inescapable novelty test and the amphetamine sessions was measured in a locomotor chamber, measuring $40.64 \times 40.64 \times 40.64$ cm (Coulbourn Instruments, TruScan 2.01). The chamber consisted of plexiglass walls and plastic flooring which was covered with pine chip bedding using procedures described previously (Arndt et al., 2014; Cain et al., 2012; Garcia et al., 2015; Garcia and Cain, 2016).

2.4 Treatment

D-amphetamine (Sigma Aldrich Dallas, TX, USA) was dissolved in 0.9% saline (Experiment 1: 0.5 mg/kg; Experiment 2: 0.3 mg/kg).

2.5 Procedure

2.5.1 Experiment 1: Differential housing and change in novelty response—

Sixty rats arrived in the laboratory at post-natal day (PND) 21. They were housed in pairs for approximately 24 hours after arrival. The morning after arrival, rats were tested for their response to inescapable novelty (IEN). Rats were weighed and transferred to the testing room and placed in locomotor chambers for 1h. Locomotor activity was measured by recording the total distance traveled (cm). After the initial IEN test, to ensure that there were not baseline differences in novelty response between the housing conditions, a median split of the distance traveled (cm) during the IEN test was used to classify rats as High (HR) or Low (LR) responders. We then equally divided the rats based on their novelty response across the environmental housing conditions. The median split group assignments were used only to ensure a relatively equal distribution of responses on the IEN test across the differential housing assignments and not for any statistical analyses. Prior to the start of the dark cycle on PND 22, an equal number of higher and lower novelty responding rats were placed in the EC (10 HR, 10 LR), IC (10 HR, 10 LR), or SC (10 HR, 10 LR) for a 30 day housing period. At PND 53 day, rats were again tested for their response to IEN using the procedures described above (Figure 1A). To maintain the novelty of the locomotor chambers, compressed pellet bedding was used on the floors and the walls were covered with novel black and white geometric patterns.

2.5.2 Amphetamine-induced hyperactivity—After the second novelty test, at PND 53, rats received repeated injections of amphetamine or saline. Rats were randomly assigned to the amphetamine (n=10 EC, n=10 IC, n=10 SC) or saline (n=10 EC, n=10 IC, n=10 SC) condition. Rats received repeated injections of d-amphetamine sulfate (0.5 mg/kg, s.c) or saline immediately prior to being placed in locomotor chambers for seven 1h sessions every other day. On alternating rest days, they remained in their home cages and received no injections. Following seven sessions, rats remained in their home cages and did not receive any injections for the next 14 days. Rats then received an amphetamine injection (0.5 mg/kg, s.c) before being placed in the locomotor chambers for 1h to test for sensitization.

2.6 Procedure Experiment 2: Differential housing and novelty response

2.6.1 Phase 1: Effects of housing conditions on response to novelty and amphetamine—Forty-eight rats were randomly assigned to the EC (n=24) or IC (n=24)

housing upon arrival in the laboratory at 21 days of age. Following the 30 day housing period, rats were tested for response to novelty (IEN) using the same procedures described in Experiment 1. Rats were then randomly assigned to either an amphetamine (0.3 mg/kg; s.c.) or saline group. Immediately prior to five 1h locomotor sessions, rats received their assigned injection of amphetamine or saline. On alternating rest days, they remained in their home cages.

2.6.2 Phase 2: Effects of switching housing conditions in adulthood on responses to novelty and amphetamine—At approximately PND 63, 24 rats were switched to the opposing environment (EC to New IC n=12; IC to New EC n= 12) while the other 24 rats remained in their original environment (EC n=12; IC n=12). The rats remained in their new or original conditions for 30 additional days (Figure 1B). Following the second 30 day period, approximately PND 93, rats were again tested for response to novelty (IEN). The procedures used during the initial novelty exposure were used with the exception that the bedding (compressed pellet bedding) and walls (black and white geometric pattern) of the chamber were changed to ensure the chamber was novel. After the second novelty test, rats remained in their same amphetamine or saline groups and the locomotor response to amphetamine or saline was measured during 5 sessions. Rats were administered their assigned injection of amphetamine or saline (0.3 mg/kg; s.c.) and immediately placed inside the locomotor apparatus for 1h. On rest days rats remained in their home cages. Then, rats were given a 14-day rest period followed by an injection of amphetamine (0.3 mg/kg; s.c.) and locomotor activity was measured for 1-hr to test for behavioral sensitization.

2.7 Data Analysis

The current study used statistical techniques founded in the General Linear Model to determine if housing environment changed the response to novelty and locomotor response to amphetamine. Therefore, we used a mixed factorial ANOVA in Experiment 1 to determine how locomotor activity changed across time in response to amphetamine or saline in differentially housed rats (housing group (EC/IC/SC) $\times$ treatment (amp/sal) $\times$ session). A similar mixed factorial ANOVA was used for phase 1 of Experiment 2 (EC/IC $\times$ amp/sal $\times$ session). In phase 2 after the switch, a separate mixed factorial ANOVA, housing group (EC/EC_{New}/IC/IC_{New}) $\times$ treatment (amp/sal) $\times$ session) examined differences in locomotor activity. To fully examine the ANOVA results, planned comparisons of the main and interaction effects were probed using simple effects.

In addition to the ANOVA analyses, another important aim of the study was to examine individual differences in response to novelty, because of the increased vulnerability to psychostimulant addiction in high novelty responders. Therefore, moderated-mediation analyses were used to determine if housing-induced changes in response to novelty could account for housing group differences observed in locomotor response to treatment. Moderated-mediation analysis offer the ability to understand how one variable can partially or fully account for the variance in the criterion. This statistical approach assesses more than R^2 changes and identifies whether the inclusion of a variable accounts for variance above and beyond another variable in the model (Hayes, 2013). Moderated-mediation analysis models the total effect of a predictor (X) on a criterion (Y) both directly, and indirectly

through a mediator (M). Mediation is indicated when the mediator (M) significantly predicts the criterion (Y), and the predictor (X) no longer significantly predicts the criterion (Y) when the mediator (M) is included in the model. Further, an additional variable (V) can change (moderate) the mediation effect by which the mediator (M) may explain the relationship between the predictor (X) and the criterion (Y). For these moderated mediation models in the context of our experiments, the predictor (X) is the housing group, the mediator (M) is the locomotor response during IEN test, the moderator (V) is the treatment (amphetamine or saline), and the criterion (Y) is the distance traveled after treatment either acutely or during the sensitization test. Direct, indirect, and conditional indirect effects were assessed using *t*-tests and confidence intervals. Confidence intervals estimate the true effect in the population from the results of our experiments. If the estimate of the population effect, as indicated by a 95% confidence interval, did not contain 0, then we would be 95% confident that the true population effect is different from 0. All alpha levels were set at $p < .05$. Mediation analyses were completed with PROCESS, a free SPSS plug in written by Dr. Andrew Hayes (Hayes, 2013).

3. Results

3.1 Experiment 1: Effect of housing environment on novelty response

The initial IEN test occurred prior to housing assignment. The environmental housing condition did alter the response to inescapable novelty during the second IEN test (Figure 2). The mixed factorial ANOVA revealed main effects of time, $F(1, 57)=108.31, p<.001$, housing condition, $F(2, 57)=43.99, p<.001$, and a significant interaction, $F(2, 57)=33.62, p<.001$, suggesting the response to novelty was changed differently by housing condition. Bonferroni multiple comparisons revealed that all three housing conditions differed from each other during the second IEN tests. IC rats showed a greater response to novelty at test 2 when compared to SC, $F(1, 38)=8.93, p<.01$, and EC rats $F(1, 38)=158.38, p<.001$, and SC rats showed a greater response to novelty at test 2 when compared to EC rats $F(1, 38)=96.46, p<.001$.

3.2 Housing condition alters the behavioral response to repeated amphetamine

A (housing condition $\times$ treatment $\times$ session) mixed factorial ANOVA revealed significant main effects of housing condition, $F(2, 54)=19.22, p<.001$, treatment, $F(1, 54)=245.74, p<.001$, and session, $F(6, 324)=7.09, p<.001$. Tests of interactions indicated that all 2-way interactions were significant in addition to a significant 3-way interaction between housing condition $\times$ treatment $\times$ session, $F(12, 324)=2.86, p<.001$. These results indicated that amphetamine significantly increased locomotor activity compared to saline treatment. While SC rats had greater amphetamine-induced locomotion than did EC and IC rats across all sessions, EC and IC rats did not differ, which did not support our hypotheses; we hypothesized that EC housing would reduce locomotor activity compared to IC and SC housed rats. A housing condition $\times$ treatment factorial ANOVA revealed main effects of housing condition, $F(2, 54)=15.46, p<.001$, and treatment, $F(1, 54)=13.29, p<.001$, on locomotor response to amphetamine on the sensitization test day. Planned comparisons for the sensitization test revealed, only the SC rats previously administered amphetamine showed locomotor sensitization via an increased locomotor response compared to SC rats

previously administered saline, $F(1, 54)=12.81, p<.001$). EC, $F(1, 54)=3.20, p>.05$ and IC $F(1, 54)=0.89, p>.05$ rats did not show locomotor sensitization, which partially supported our hypothesis that EC housing would protect against locomotor sensitization, but the lack of sensitization in IC rats does not fully support the hypothesis that IC housing increases the vulnerability of locomotor sensitization (Figure 3).

3.3 Discussion Experiment 1

The current results provide evidence that the differential housing paradigm alters the locomotor response to novelty. The data indicate that PND period between days 21–51 represents an important time period for the development of novelty and sensation seeking. High adolescent novelty seeking and young adulthood is known to predict drug experimentation and poly-drug use (Wellman et al., 2014). Therefore, our results suggest that differential housing may be altering the novelty response at a critical time that is important for drug experimentation and acquisition.

SC rats showed greater locomotor activity to amphetamine when compared to EC and IC rats. Further, the SC rats demonstrated behavioral sensitization, while the EC and IC rats did not. This result is consistent with previous research suggesting that at moderate to high doses of amphetamine, the differences between EC and IC rats may be diminished (Arndt et al., 2014; Bardo et al., 1995; Cain et al., 2012; Gill et al., 2012; Wooters et al., 2011). We speculate that with high doses the locomotor stimulating effects of amphetamine surmount the protective effect of an enriched environment.

3.4 Experiment 2: 0.3 mg/kg amphetamine

Experiment 2 was designed to test our hypotheses that PND 21–51 are critical for the development of novelty and sensation seeking and differences between EC and IC rats are more robust at lower amphetamine doses. To test these hypotheses, we implemented two housing periods; the first occurred predominately in adolescence and the second occurred in adulthood (Spear, 2000); see Figure 1B for details). We predicted that differential housing conditions would result in differences in the novelty response after adolescence, but that the novelty response would remain stable in adulthood. To test our hypothesis that differences between EC and IC rats are more robust at lower doses, we used 0.3 mg/kg dose of amphetamine.

3.4.1 Phase 1: Inescapable novelty test before switch—After the initial housing period, IC rats had more locomotor activity compared to EC rats during the IEN test, $F(1, 47)=124.17, p<.001$, indicating a greater response to novelty (Figure 4). This result supports the first part of our hypothesis that novelty and sensation seeking has a critical developmental period in adolescence and early adulthood, and replicates the results from Experiment 1.

3.4.2 Repeated amphetamine exposure—When using the low unit dose of amphetamine, EC housing decreased amphetamine-induced hyperactivity. The $2 \times 2 \times 5$ mixed factorial ANOVA revealed main effects of housing condition, $F(1, 44)=108.13, p<.001$, treatment, $F(1, 44)=97.77, p<.001$, and session, $F(4, 176)=6.41, p<.001$. The ANOVA also

revealed significant interactions between session and housing condition, $F(4, 176)=5.27$, $p<.001$, such that only IC rats showed an increase in locomotor activity after repeated administrations of amphetamine. There was also a significant interaction between session and treatment, $F(4, 176)=7.11$, $p<.001$. The housing condition $\times$ treatment $\times$ session interaction was trending toward significance, $F(4, 176)=2.08$, $p=.08$.

3.5 Phase 2: Effects of switching environmental conditions in adulthood on responses to novelty and amphetamine

The next phase was designed to test the hypothesis that adolescence and early adulthood are critical developmental periods for the novelty response. In accord with our results, we hypothesized the novelty response would change during the first housing manipulation which occurred predominately during adolescence, but would not change after the second housing manipulation in adulthood.

3.5.1 Response to novelty after the environmental switch—Switching the housing condition significantly altered the response to inescapable novelty test, and suggested the novelty response is malleable into adulthood, $F(3, 44)=33.77$, $p<.01$ (Figure 4). EC rats had significantly less locomotor activity compared to IC rats, $F(1, 44)=83.48$, $p<.05$, and New IC rats, $F(1, 44)=22.02$, $p<.05$. Rats once in the IC condition but now in the EC (New EC) did not show any significant differences in response to novelty compared to rats that remained in the EC condition. New IC rats had less locomotor activity compared to IC rats that remained in the IC condition throughout the entire experiment, $F(1, 44)=19.75$, $p<.05$. Also, New EC rats had less locomotor activity compared to IC rats that remained in the IC condition throughout the entire experiment, $F(1, 44)=63.56$, $p<.05$. Taken together these results indicate the novelty response remains malleable well into adulthood and that differential housing is a robust manipulation to change the response to novelty.

3.5.2 Locomotor response to treatment across phases—To determine if switching the housing conditions in adulthood altered the response to amphetamine within groups, we compared responding during the last session of Phase 1 to responding during the first session of Phase 2. The ANOVA revealed main effects of environmental condition, treatment, and a significant interaction between session and environmental condition, $F(3, 40)=15.08$, all $ps<.001$, (Figure 5A and 5B), indicating that remaining in the EC condition was most beneficial for attenuating the response to amphetamine. Further, IC rats switched to EC showed an attenuation in response to amphetamine.

3.5.3 Repeated Amphetamine Exposure—A (housing condition $\times$ treatment $\times$ session) mixed factorial ANOVA for Phase 2 revealed main effects of housing condition, $F(3, 39)=23.92$, $p<.001$, treatment, $F(1, 39)=201.51$, $p<.001$, and session, $F(5, 195)=27.84$, $p<.001$, and significant interactions between session and housing condition, $F(15, 195)=2.13$, $p=.01$, and session and treatment, $F(5, 195)=23.92$, $p=.001$ (Figure 6A & 6B). The three way interaction was trending toward significance, $F(15, 195)=1.62$, $p=.07$. Planned simple effects indicated that rats that were switched from EC to IC housing lost the protective effect from EC housing as evidenced by the significant difference between EC and New IC after repeated amphetamine, particularly after the third amphetamine exposure

$F's(1, 156)=15.00-41.72, p's<.001$. Importantly, EC housing in adulthood was able to rescue the rats from adolescent IC housing, as evidenced by the differences between IC and New EC rats $F's(1, 156)$ range 6.47–44.77, $p<.001$. To determine if switching the housing conditions in adulthood altered amphetamine-induced sensitization, all rats were administered amphetamine following a 14 day rest period. Simple effects indicated that EC rats continuously housed in enrichment had less locomotor activity when compared to IC rats $F(1, 39)=5.35, p<.05$, when previously administered amphetamine. When compared to the previously saline treated rats, both the EC housing conditions expressed locomotor sensitization on the test day, EC $F(1, 39)=8.87, p<.001$; New EC $F(1, 39)=4.53, p<.05$). However, this effect is likely driven by the low amount of locomotor activity in the EC and New-EC rats previously administered saline (Figure 7).

3.6 Discussion Experiment 2

Our hypothesis that novelty response in late adulthood is stable was not supported. The results from Experiment 2 indicate two important findings. First, the novelty response does not have a single critical developmental period that occurs *only* during adolescence and early adulthood. More likely, the response to novelty appears to be malleable well into adulthood. The malleability of novelty well into adulthood is important because while novelty and sensation seeking is a risk and vulnerability factor for drug experimentation and compulsive drug taking in adolescence (Chambers et al., 2003; Spear, 2000), it is also a risk factor in adulthood (Hutchison et al., 1999; Kelly et al., 2006; Kirkpatrick et al., 2013; Sax and Strakowski, 1998). Therefore, we propose that an augmented novelty response is risky in adolescence or adulthood. Our results indicate that there is a relationship between adult novelty response and the locomotor response to amphetamine.

The other finding is that housing environment is a manipulation capable of changing the novelty response well into adulthood. This study provides evidence that behavioral interventions targeted at changing the novelty response could be useful in blunting the locomotor response to amphetamine, particularly when low-unit doses are used. Therefore, the ability of enriched housing to rescue rats previously housed in isolation, suggests novel, complex, and socially stimulating environments may be a possible intervention to change risk vulnerabilities or traits. Our experimental design allows us to test the hypothesis that novelty response can account for locomotor response above and beyond housing environment. To fully determine if the altered novelty response is changing the response to amphetamine or saline, we conducted a moderated mediation analyses. This analysis tests whether the mediating variable (IEN response) can partially or fully account for differences in the locomotor response to amphetamine or saline.

4. Mediation analyses

The moderated mediation analyses focused only on the results from Experiment 2, because the most robust differences between EC and IC rats were identified at the 0.3 mg/kg dose of amphetamine. We also aimed to determine if the adolescent or adult novelty response was more important for the initial response to amphetamine. Therefore, we focused the moderated mediation analysis on the first treatment of amphetamine or saline after the

housing period in both Phases 1 and 2, because the effect of housing period on novelty and locomotor response would theoretically be the strongest. Model “One”, modeled the effects of the adolescent housing period (Phase 1), and model “Two” modeled the effects of the adult housing period (Phase 2). We hypothesized that the novelty response would mediate the locomotor response to amphetamine or saline, and the strength of the mediation effect would be larger in adolescence. We hypothesized this because most drug abuse begins in adolescence, and novelty seeking is higher in adolescence (Chambers et al., 2003; Spear, 2000; Stansfield and Kirstein, 2005). Therefore, we predicted the altered novelty response would show a larger positive relationship with treatment-induced locomotor activity during this developmental period. To make this comparison, we compared the standardized β coefficients and the confidence intervals. The absolute value of β coefficient allows comparison across models and if the confidence intervals do not overlap it would indicate that the novelty responses (adolescent vs adult novelty response) are different from each other.

4.1 Mediation initial amphetamine treatment– Phase 1 Session 1

This mediation model tested the direct and indirect effects of housing group on distance traveled on the 1st administration of treatment. The mediating variable is the IEN test after the initial housing period. The moderator of mediation was treatment (saline or amphetamine 0.3 mg/kg). The moderator tests whether the mediation effect is specific to saline or amphetamine treatment.

The overall mediation model for the first administration significantly predicted distance traveled in response to the first treatment, $R^2=.68$, $F(4, 43)=23.03$, $p<.001$. Housing group had a significant direct effect in predicting distance traveled, with EC rats moving less than IC rats (see above Phase 1 results for full details) $t(43)=2.46$, $p<.05$. There was evidence for the indirect effect with housing group predicting the response to novelty for the initial IEN test with the IC rats demonstrating a greater novelty response, $R^2=.72$, $F(1, 46)=124.17$, $p<.001$. However the initial IEN test did not predict distance traveled on day 1, providing no evidence of an indirect relationship and therefore the initial IEN test cannot mediate housing group’s prediction of distance traveled (Figure 8). Furthermore, neither amphetamine nor saline altered the prediction of the IEN test, providing more support that there was not a mediation effect occurring (95% CI [-378.09, 3,174.13] and [-1,882.61, 4,491.36], respectively). Our hypothesis regarding a positive relationship between novelty response and initial amphetamine response was not supported, and suggests that adolescent novelty response does not indirectly affect the locomotor response to amphetamine at a low-unit dose.

4.2 Mediation description phase 2

The IEN test after the housing condition switch was now identified as the mediator variable. These mediation analyses tested the direct and indirect effects of housing group on treatment-induced locomotor activity on day 1 of phase 2. Like before, the treatment—amphetamine or saline—was treated as a potential moderator of the mediation of the 2nd IEN test. Again, we hypothesized that housing condition would change the novelty response and that novelty response would change the response to amphetamine or saline, such that

higher novelty would result in greater locomotor activity in response to amphetamine or saline. We, however, thought this relationship would be smaller than the predictive relationship in the first mediation model.

4.2.1 Initial amphetamine treatment– Phase 2 Session 1—The mediation model predicting the distance traveled on the first administration after the 2nd housing period accounted for a significant amount of variance, $R^2=.72$, $F(4, 43)=27.36$, $p<.001$. After the switch, there was a direct effect of housing group on the locomotor response to amphetamine or saline, such that continuous EC housing resulted in the least response and continuous IC housing resulted in the greatest response. The two switches were not different from any groups (see above Phase 2 for details). However, after testing the indirect effects, the once significant direct path of housing group no longer had a direct effect on distance traveled during session 1 treatment administration, $t(43)=0.20$, $p>.05$. Testing the indirect path, the housing group did significantly alter the response to novelty, $R^2=.33$ ($F(1, 46)=23.13$, $p<.001$). The indirect effect of the 2nd response to novelty predicted the distance traveled and suggested a mediation effect, $t(43)=2.86$, $p<.01$ (Figure 9). Housing-induced changes in the response to novelty can fully account for distance traveled in response to treatment after an environmental switch. Examination of conditional indirect effects to determine if this mediation effect depended on amphetamine or saline treatment determined that both saline (95% CI [300.30, 1,635.79]) and amphetamine (95% CI [461.30, 1,866.93]) showed large positive effects, suggesting no interaction. This result suggests that the IEN response is predicting the distance traveled regardless of treatment condition. Treatment significantly predicted the distance traveled on session 1 after the switch, $t(43)=2.40$, $p<.05$. In Phase 1, after the adolescent housing period the novelty response was indeed changed by the housing environment, but the altered novelty response did not account for differences in locomotor activity following treatment. However, in phase 2 after the adult housing period, the novelty response was again changed. The adult novelty response fully accounted for differences in locomotor activity following treatment, indicating an indirect (mediation) effect. The mechanism by which differential housing changes the locomotor response to treatment only depends on novelty response in adulthood. We did not predict this effect. We hypothesized the adolescent housing period would have a larger mediation effect when compared to the novelty response. Comparison of the novelty response confidence intervals from model 1 and model 2 further supports that novelty response in adolescence and adulthood are separate behaviors, which replicates previous literature (Mathews et al., 2010; Spear, 2000).

5. Discussion

The purpose of the current experiments was to determine if manipulating the novelty response using differential housing environments changes the locomotor response to amphetamine. The results indicate housing condition alters the response to novelty. These housing-induced changes to novelty can fully account for locomotor response in adulthood, suggesting novelty response is responsible for the locomotor response to amphetamine or saline. Accordingly, our research suggests that novelty response can be changed by environmental condition late into adulthood and enrichment housing reduces amphetamine-

induced hyperactivity at low-unit doses. Conversely, isolated housing results in greater amphetamine-induced hyperactivity. We propose the manipulated novelty response accounts for differences in amphetamine-induced hyperactivity.

High novelty responders show greater behavioral sensitization with repeated administration of psychostimulants, indicating they have increased drug abuse vulnerability (Hooks et al., 1991; Hooks et al., 1992). This is particularly troubling because high novelty seekers in humans try drugs of abuse at earlier ages and drop out of treatment more frequently (Cloninger, 1987; Galizio and Stein, 1983; Helmus et al., 2001; Zuckerman, 1986). Therefore, novelty response is of great interest because it not only represents a behavioral phenotype that is a risk factor for drug experimentation, but it is also a risk factor for drug abuse after frequent use. A critical component to the novelty response is the rate of habituation to the novel environment. Intrasection habituation is hypothesized to be a measure of behavioral adaptability (Leussis and Bolivar, 2006) and therefore the ability of differential housing to alter the novelty response may be due to alterations in the habituation rate. For example, IC rats may display greater locomotor activity during the IEN test due to a slower rate of habituation. While the current experiment did not explore the relationship between novelty seeking, habituation, and differential housing, future experiments should be designed to explore this relationship as recent literature suggests that habituation rate predicts a variety of clinical outcomes, including addiction (Lloyd et al., 2015).

Given the amount of evidence that supports that enrichment reduces amphetamine-induced hyperactivity, we hypothesized that housing condition would reduce the novelty response, and that the reduced novelty response would account for amphetamine induced hyperactivity. Generally, our hypotheses were supported. Most interesting, is that rats initially housed in isolation then moved to enrichment in phase two were rescued from the damaging effects induced by isolated housing. Further supporting this idea, rats housed in enrichment, then placed into isolation—a manipulation that increased the novelty response, demonstrated an augmented locomotor response to amphetamine and saline when compared to rats in continuously enriched housing. The result that the novelty response was changeable into adulthood was surprising, but clearly demonstrates that housing environment directly changes the response to novelty and amphetamine. The ability to increase and decrease the novelty response demonstrates that enrichment housing is a robust manipulation to induce behavioral changes well into adulthood even after previous amphetamine exposure. Our data provide evidence that the isolation and standard housing increases the inescapable novelty response, and may increase the likelihood for the development for behavioral sensitization; a hallmark identified as a possible feature to the transition to addiction (Robinson and Berridge, 2001).

Based on the current results, it is not clear if a period of enrichment during adulthood would be effective when a high dose of amphetamine is administered. Our experiments used a moderate dose of amphetamine in Experiment 1 (0.5 mg/kg) and low dose in Experiment 2 (0.3 mg/kg) because enrichment is most effective at preventing amphetamine-induced hyperactivity and sensitization at low-unit doses (Bardo et al., 1990; Bardo et al., 1995; Cain et al., 2012; Gill et al., 2012). Evidence from experiment 1 confirms this dose dependent relationship, and suggests that a manipulated novelty response can only reduce

amphetamine-induced locomotor activity at low unit doses. This observation is important because novel stimulating environments may be able to reduce early drug experimentation when low doses are used, but may not provide added benefit once binge-intoxication use develops. However, little evidence has emerged to fully determine if there is an interaction between amphetamine dose and enrichment length, such that longer periods of enrichment during adulthood may be necessary when higher doses of amphetamine are tested. Similarly, from our results, it is not clear if a shorter period of enrichment during adulthood would also be effective. The current study used a 30 day housing period, because previous research determined that shorter periods of enrichment did not attenuate cocaine-induced sensitization (Solinas et al., 2008). Switching to enrichment following isolation decreased both acute and repeated amphetamine-induced hyperactivity, however, placement in enrichment during adulthood was not as effective as continuous enriched housing. Therefore, a longer period of enrichment may be required to observe a longer lasting mitigation of amphetamine-induced locomotor activity, especially in adulthood or after previous amphetamine exposure. A complete dose response and housing length interaction needs to be characterized to fully determine the critical periods that contribute to protection and vulnerability.

A number of experiments have examined the effects of environmental enrichment as an intervention, such that enrichment is introduced after drug exposure or drug self-administration (Grimm et al., 2008; Halbout et al., 2014; Thiel et al., 2009). The results are mixed, such that enrichment may attenuate cue-reinstatement, but not cocaine or sucrose reinstatement. However, our results suggest that housing in enrichment throughout adolescence is most beneficial, and enrichment following isolation is better than remaining in isolation. Although the New IC rats showed increases in locomotor response when compared to the permanent EC rats, the New IC rats did not show the same augmented response to amphetamine that the permanent IC rats did. Taken together, the results suggest that the most robust effects of enrichment housing occur when animals are housed in enrichment and maintained in enrichment. Significant differences between EC, IC, and SC rats were present following saline treatment in Experiments 1 and 2.

It is possible that the amphetamine-induced differences between EC, IC, and SC rats are the result of differences in control rates of behavior using the rate dependency theory (Dews and Wenger, 1977). However, interpreting the data within the theoretical framework of rate dependency can not only change the magnitude of the effect, but also the direction of the effect (Smith et al., 2009). Rate dependency is correlated and confounded with reinforcement schedule (Lucki, 1983), but the current experiment measured non-contingent behavior. Therefore, there was no contingent relationship between the locomotor response to amphetamine or saline and a reinforcer, suggesting that rate dependency does not explain the effects of differential rearing on amphetamine-induced hyperactivity. In Experiment 1 locomotor activity between EC and IC rats did not differ following the low dose of amphetamine when differences were present in the saline condition. This result is consistent with several papers demonstrating that EC and IC rats do not differ in response to low and moderate doses of amphetamine (Arndt et al., 2014; Bardo et al., 1995; Bowling and Bardo, 1994; Cain et al., 2012; Gill et al., 2014; Gill et al., 2012), and suggest that the locomotor differences observed following amphetamine are not solely due to rate dependency.

Previous research has determined that enrichment alters the response to novelty (Fuller, 1967; Lore, 1966; Simpson and Kelly, 2011), and our results are in agreement with these studies, and importantly, that enrichment during adulthood also alters the adult novelty response. The literature examining novelty response and drug vulnerability has determined that increased novelty response increases psychostimulant-induced hyperactivity and high novelty responders self-administer more psychostimulants (Bardo et al., 2013; Flagel et al., 2014; Kabbaj, 2006; Piazza et al., 1989; Piazza et al., 1990). The neurobiological alterations that result from the housing manipulation are likely in the mesoaccumbens circuitry because the ventral tegmental area, nucleus accumbens, and ventral pallidum are all implicated in the novelty response and the expression of amphetamine-induced locomotor activity. Future research should identify this circuit to determine if the housing manipulation is directly altering the function of dopamine and GABA neurotransmission within this circuit (Hooks and Kalivas, 1995). The current experiment did not examine if a similar relationship exists between housing condition and novelty during psychostimulant self-administration. An extensive literature indicates that the novelty response predicts the acquisition of low unit doses of psychostimulants (Piazza et al., 1989; Piazza et al., 1990), and that enrichment decreases the self-administration of low unit doses of amphetamine (Arndt et al., 2015; Bardo et al., 2001). Therefore, we hypothesize that a similar mediation pattern between novelty and housing conditions would remain during self-administration of low unit doses. Future experiments will test this hypothesis and also examine the relations when higher doses of amphetamine are used. Our results from the current experiment suggest that enrichment during adulthood may be an effective strategy to decrease the response to novelty and psychostimulants, and therefore reduce drug abuse vulnerability in adolescence and adulthood.

6. Conclusion

The present results suggest that placement in enrichment during adulthood can attenuate the locomotor response to amphetamine, even in rats originally housing in isolation. These results provide support that enrichment during adulthood can be used as an intervention to reduce the novelty response and locomotor response to psychostimulants. Further, different housing conditions in adulthood can change the effect of early enrichment. Taken together, differential housing during adolescence and differential housing during adulthood can substantially alter the response to novelty and sensitivity to amphetamine, but the adult novelty response may be more important for the development of amphetamine-induced hyperactivity—a behavioral measure of critical adaptations for the development of drug addiction.

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Abbreviations

EC	Enriched condition
IC	Isolated condition
SC	Standard condition
IEN	Inescapable novelty test
PND	Post-natal day

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Highlights

- Isolation and standard housing increase the novelty response
- Higher novelty response resulted in greater amphetamine-induced hyperactivity
- Enrichment reduces the novelty response and blunts amphetamine-induced hyperactivity
- Switching isolated rats to enriched reduced novelty and amphetamine responses
- Housing-induced changes to novelty mediate amphetamine-induced hyperactivity

A**Experiment 1** **B****Experiment 2** **Fig. 1.**

Timeline to illustrate the experimental design for experiments one (A) and two (B). For experiment two, the grey boxes represent Phase 1 of the study. The black boxes represent Phase 2 of the study. In Phase 2, half of the animals in each environmental condition were switched to the other environmental condition (New EC & New IC). They were housed in these conditions for an additional 30 days before experiments commenced.

Fig. 2.

The mean ($\pm$ SEM) response to inescapable novelty (IEN) measured in total distance traveled (cm) for EC, IC, and SC rats. There were no differences in novelty response at IEN 1. After housing, there were significant differences between all housing groups (*) at IEN 2. (* indicate $p < .05$ or less).

Fig. 3.

The mean ($\pm$ SEM) total distance traveled (cm) on the sensitization test session. All rats received amphetamine (0.5 mg/kg). SC rats previously treated with amphetamine showed greater locomotor response (#) when compared to SC rats previously treated with saline. SC rats treated with amphetamine showed greater locomotor response (*) when compared to EC and IC rats. (*, # indicate $p < .05$ or less).

Fig. 4.

The mean ($\pm$ S.E.M.) total distance traveled (cm) during the inescapable novelty test after the initial housing period (IEN 1) and after the switch (IEN 2). A significant difference between EC and IC rats was observed on the first IEN test (*). At the second IEN test all groups were different except the EC and New EC groups. Asterisks (*) indicate significant differences between housing groups (all p 's $< .005$).

Fig. 5.

Fig. 5A and 5B. The mean ($\pm$ S.E.M.) total distance traveled (cm) during final session during Phase 1 and the first session during Phase 2 in rats that received amphetamine (A) or saline (B). Rats previously housed in the IC condition and switched to the EC condition

(New-EC) had a decrease in locomotor activity (*). Animals previously housed in the EC condition and switched to the IC condition (New-IC) had an increase in locomotor activity (^). (*, ^ indicate $p < .05$ or less).

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Fig. 6.

Fig. 6A and 6B. The mean ($\pm$ S.E.M.) total distance traveled (cm) during sessions 1–5 of Phase 2 for rats administered amphetamine (A) or saline (B). Asterisks (*) indicate a significant difference between EC and New-IC rats (during sessions 1, 3–5 for

amphetamine-treated rats). Numerical signs (#) indicate a significant difference between IC and New-EC rats. Ampersands (&) indicates a significant difference between sessions 1 and 5 for amphetamine treated IC and New-IC rats. (*, #, ^, & indicate $p < .05$ or less).

Fig. 7.

Mean (+/- SEM) total distance traveled (cm) on the sensitization test day. All rats received amphetamine 0.3 mg/kg for the test. Black amphetamine bars represent rats previously treated with amphetamine. White horizontal striped bars represent rats previously treated with saline. * indicates difference between amphetamine and saline. ^ indicates a significant difference from other groups previously treated with amphetamine. # indicates a significant difference from rats previously treated with saline. (*, #, ^ indicate $p < .05$ or less)

Fig. 8.

Conceptual moderated mediation model for experiment 2 Phase 1. β represents the standardized unique relationship between the two variables. Treatment refers to amphetamine or saline treatment. After the initial housing period, housing group significantly predicted the distance traveled in response to treatment on day 1. Housing group also predicted the response to novelty. The response to novelty did not predict distance traveled providing no evidence for mediation. Amphetamine increased distance traveled. (*) indicates a significant relationship between two variables.

Fig. 9.

Conceptual moderated mediation model for experiment 2 Phase 2. β represents the standardized unique relationship between the two variables. Treatment refers to amphetamine or saline treatment. After the environmental switch, housing group predicts distance traveled in response to amphetamine or saline on day 1 and on the sensitization test day. Housing environment also predicts the response to novelty in adulthood. When the response to novelty is included, housing environment no longer predicts distance traveled on day 1. (*) indicates a significant relationship between two variables.